

## Chemical Blood Studies.\*

### V. Comparative Studies on "Laked" and "Unlaked" Blood Filtrates of Bovines in Health and during Anaplasmosis (*A. marginale* infection) and Piroplasmosis (*P. bigeminum* infection).

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## I.—INTRODUCTORY NOTE.

The present paper is one of a series of publications on researches into the composition of the blood of domestic animals, both in health and during the course of various infections, (stock diseases), occurring in South Africa. It embodies the results of the experimental work performed in connection with anaplasmosis and piroplasmosis. The aims, plan of research, methods of analyses used and general technique, arrangement of data, etc., have been fully set out in the first paper [Chemical Blood Studies I., page 269, of this Journal and in order to avoid needless repetition this aspect of the work has been omitted here.

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## II.—ANAPLASMOSIS OF CATTLE.

### (a) SYMPTOMATOLOGY AND PATHOLOGICAL ANATOMY.

The disease investigated is that produced in susceptible animals by an infection with *Anaplasma marginale*.

The condition is briefly described here from a symptomatological and pathological-anatomical point of view for the purposes of a clearer understanding of the correlation between the chemical blood changes and the disease complex. A bibliography, listing some of the more important publications on anaplasmosis, will be found at the end of this article. No references could be found in the available literature to any chemical researches into this condition.

Anaplasmosis has been defined (Theiler, 1910) as a disease of cattle, caused by a protozoön, *Anaplasma marginale*, which invades and destroys the red blood corpuscles causing primarily an acute oligocythaemia accompanied by high fever and secondly, a degeneration of all parenchymatous organs. Recovery from the disease gives resistance to subsequent infections. The immune animal acts as a reservoir for the virus and the blue tick (*Boophilus decoloratus*) acts as host or transmitter of the parasite." Subsequently various other ticks have been found capable of transmitting the disease.

The disease (Knuth and du Toit, 1921) can also be transmitted artificially through the subcutaneous, intravenous and intramuscular injection of infected blood into susceptible bovines.

The incubation period varies considerably, e.g. from 16–17 days by sub-inoculation, and 60–80 days with tick transmission. A fever reaction is usually the first symptom, followed by loss of appetite, loss of condition, anaemia, icterus, salivation and generally constipation, rarely diarrhoea. In severe cases the patient goes down. The urine is icteric, but very rarely blood stained. There is dyspnoea, an increased heart rate, and frequently muscular tremblings shortly before death. The severity of the symptoms vary considerably. They may be so mild as to virtually escape detection except for a slight fever reaction, or the patient may show all or most of the above symptoms, especially imported cattle. Calves show as a general rule only relatively mild symptoms.

Pathological-anatomically the following changes are usually noted: generalised icterus, gelatinous transudation into the tissues of the neck, sternum and abdomen; severe anaemia, and oligocythaemia, the blood being thin, watery and staining badly; subepicardial and occasionally subendocardial haemorrhages; tumor hepatitis and yellow coloration due to bile stasis and fatty degeneration; enlarged gall bladder containing a viscid thick dark green bile; tumor splenis and subcapsular haemorrhages and impacted omasum.

Morphologically the blood, in addition to the presence of parasites, reveals marked anaemic changes, such as anisocytosis, polychromasia, basophilla, uncleated red cells (Jolly bodies) and a decreased erythrocyte count. In spite of severe erythrocyte destruction, haemoglobinuria very rarely occurs. The severity of the blood changes may vary from a slight anisocytosis in mild cases, to the above described changes in severe reactions.

#### (b) METHODS FOR ANALYSIS AND TECHNIQUE.

The same methods and technique as previously described in paper I of this series (p. 000), was adhered to. Bovine blood tends to disintegrate more rapidly during the preparation of "unlaked" filtrate, and as soon as sufficient filtrate has been obtained, the filter paper should be removed. This is particularly the case with pathological bloods.

#### (c) EXPERIMENTAL DATA IN ANAPLASMOSIS.

The analytical figures submitted here were obtained from 9 cases of anaplasmosis, of which 3 proved fatal, 4 could be regarded as severe, and 2 as very mild. The transmission was always by means of subcutaneous inoculations of 5-10 c.c. infected blood, the animals being placed on temperature, blood smears examined frequently, and analyses made throughout the course of the disease. In all cases a "pure" strain of *A. marginale* was utilised, i.e. uncomplicated by the presence of the redwater parasite (*P. bigeminum*). In some cases chemotherapeutic treatment by means of subcutaneous or intravenous injections of various drugs was given in an endeavour to find an effective anaplasmodicidal agent. This aspect of the problem was under the charge of Mr. Parkin of this Division, and in view of his intention to shortly publish his results on a large number of drugs tested out, I feel constrained to omit here publication of his data. I have, however, with his sanction indicated on the temperature charts the periods at which such injections were made. In a few cases a definite effect of the drug as evinced by the sudden drop in the temperature, in others no such visible effect is noticeable. Four patients were, however, untreated, and the analytical data obtained from them therefore represents the conditions as they are found in natural cases.

In the arrangement of the analytical data, the same sequence as previously used has been followed, i.e. the temperature record, a short history of the animal, symptoms shown, etc., the analytical data, followed by a brief discussion of the main abnormalities noted in the blood in each particular case. A general summary and a discussion of the findings concludes this section.

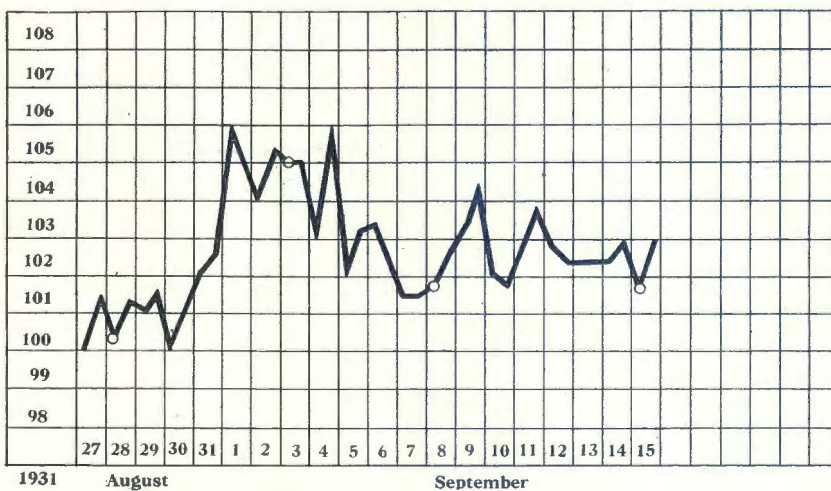
From the morphological aspect of normal bovine blood under South African conditions, I would refer to a paper by Canham (1930), in which the erythrocyte count, differential count and the influence of various factors such as exercise, pregnancy, altitude, lactation, age, etc., is discussed.



CASE I.

B. 2889. *Anaplasmosis (Treated and Recovered)*.

Temperature Chart I.



*History.*—B. 2889, a young ox (6 tooth), received here from the Government Cattle Ranch, Messina, 15/2/29, and immunised against blackquarter in May, 1929. Negative for tuberculosis (tuberculin test in June, 1931). Was drafted into the anaplasmosis experiment, 18/8/31, receiving on even date subcutaneously 5 c.c. blood from B. 3056 which at the time was reacting with an *A. marginale* infection. On 31/8/31 the first few anaplasms were noted coinciding with the beginning of the hyperthermic reaction. The parasites became frequent, and the usual blood changes associated with this infection developed. The animal did not feed well at the beginning and showed anorexia for about seven days during the main reaction; the faeces became reduced in amount and firm in consistency. Was treated, 3/9/31, chemotherapeutically. By 25/9/31, i.e. 37 days p.i., the parasites had virtually disappeared, but moderate blood changes still persisted. Made an uneventful recovery and was discharged in December, 1931.

TABLE 1.—B. 2889. *Anaplasmosis (Treated) Recovered.*

| Date.....           | 18/8/31. | 20/8/31. | 28/8/31. | 3/9/31. | 8/9/31. | 15/9/31. | 17/9/31. | 22/9/31. | 7/10/31. | 15/10/31. | 22/10/31. | 11/11/31. |
|---------------------|----------|----------|----------|---------|---------|----------|----------|----------|----------|-----------|-----------|-----------|
| Time.....           |          |          |          |         |         |          |          |          |          |           |           |           |
| Temp. Reaction..... | N        | P.I.N.   | P.I.N.   | R       | R       | R        | N        | N        | N        | N         | N         | N         |
| Haemoglobin.....    | 16.87    | 17.51    | 17.51    | 12.78   | 6.44    | 5.07     | 5.61     | 7.25     | 10.60    | 12.94     | 13.31     | 15.42     |
| Sugar mgm. %...     | L        | 82.6     | 90.9     | 52.91   | —       | 50.00    | 94.90    | 60.20    | 53.19    | 47.62     | 67.50     | 43.67     |
|                     | U        | 76.8     | 82.6     | 54.64   | —       | 40.80    | 70.00    | 41.60    | 51.00    | 33.33     | 60.00     | 37.45     |
| Total N. gm. %..... | 3.025    | 3.400    | 3.305    | 2.955   | 2.094   | 1.884    | 2.017    | 2.200    | 2.773    | 2.920     | 2.892     | 3.046     |
| N.P.N. mgm. %.      | L        | 21.55    | 20.00    | 20.00   | 21.70   | 19.11    | 22.90    | 19.86    | 10.98    | 17.44     | 18.43     | 18.13     |
|                     | U        | 13.50    | 16.13    | 13.80   | 16.21   | 15.31    | 17.34    | 14.92    | 13.35    | 12.53     | 12.53     | 12.50     |
| Coag. N. gm. N %    | L        | 3.005    | 3.378    | 3.285   | 2.935   | 1.865    | 1.994    | 2.180    | 2.752    | 2.903     | 2.874     | 3.028     |
|                     | U        | 3.011    | 3.384    | 3.291   | 2.939   | 1.869    | 2.000    | 2.185    | 2.760    | 2.907     | 2.879     | 3.033     |
| mgm. N %..          | L        | 6.40     | 8.50     | 5.72    | 7.20    | —        | —        | —        | 6.15     | 5.13      | 6.22      | 5.26      |
| " U %..             | L        | 13.44    | 17.85    | 11.97   | 15.12   | —        | —        | —        | 13.62    | 10.71     | 13.02     | 11.13     |
| Urea                | U        | 6.30     | 8.50     | 5.43    | 5.70    | —        | —        | —        | 5.80     | 5.48      | 5.82      | 4.44      |
| " U %..             | U        | 13.23    | 17.85    | 11.34   | 11.97   | —        | —        | —        | 12.18    | 11.55     | 12.18     | 9.24      |
| mgm. N %..          | L        | —        | —        | —       | —       | —        | —        | —        | —        | —         | —         | —         |
| TC %.               | L        | —        | —        | —       | —       | —        | —        | —        | —        | —         | —         | —         |
| Total Creatinine    | U        | —        | —        | —       | —       | —        | —        | —        | —        | —         | —         | —         |
| mgm. N %..          | U        | —        | —        | —       | —       | —        | —        | —        | —        | —         | —         | —         |
| " TC %.             | U        | —        | —        | —       | —       | —        | —        | —        | —        | —         | —         | —         |
| mgm. N %..          | L        | 0.43     | 0.52     | 0.44    | 0.50    | 0.28     | 0.60     | 0.38     | 0.35     | 0.53      | 0.46      | 0.52      |
| " UA %              | L        | 1.30     | 1.56     | 1.33    | 1.50    | 0.83     | 1.80     | 1.14     | 1.05     | 1.60      | 1.39      | 1.55      |
| Uric acid           | U        | —        | —        | —       | —       | —        | —        | —        | —        | —         | —         | —         |
| " UA %              | U        | —        | —        | —       | —       | —        | —        | —        | —        | —         | —         | —         |
| Amino acid          | L        | 7.20     | 6.40     | 6.50    | 6.63    | 6.93     | 9.21     | 8.38     | 5.45     | 6.90      | 5.45      | 5.38      |
| mgm. N %            | U        | 4.40     | 4.40     | 4.00    | 4.88    | 5.38     | 5.00     | 5.30     | 3.64     | 5.00      | 3.64      | 4.12      |
| Rest Nitrogen       | L        | 4.57*    | 6.13*    | 7.34*   | 5.67*   | 9.00†    | 10.43†   | 9.00†    | 6.18§    | 2.65§     | 3.64§     | 4.00§     |
| mgm. N %            | U        | 3.50†    | 3.23†    | 3.37†   | 5.63†   | —        | —        | —        | 1.68§    | 0.38§     | 0.72§     | 0.28§     |
| Plasma.....         | n.u.     | n.u.     | n.u.     | icteric | icteric | icteric  | icteric  | n.u.     | n.u.     | n.u.      | n.u.      | n.u.      |

\* Includes "Total Creatinine Nitrogen."  
 † Includes "Urea Nitrogen."  
 ‡ Includes "Total Creatinine Nitrogen" and "Uric Acid Nitrogen."  
 § Includes "Uric Acid Nitrogen."

Main Features of Analytical Data.

Hb.—A very striking decrease in Hb. content is noted during the fever reaction, the Hb. dropping from about 17 gm. to 5 gm. per cent. The erythrocyte destruction is relatively rapid, regeneration relatively slow, the original Hb. level not being quite reached eight weeks later. Haemoglobinuria was never noted, but bilirubinaemia was present during the period of acute blood destruction.

U.A.N.—Shows a decrease during the reaction and only reaches its former level 3-4 weeks after the return of the temperature to normal.

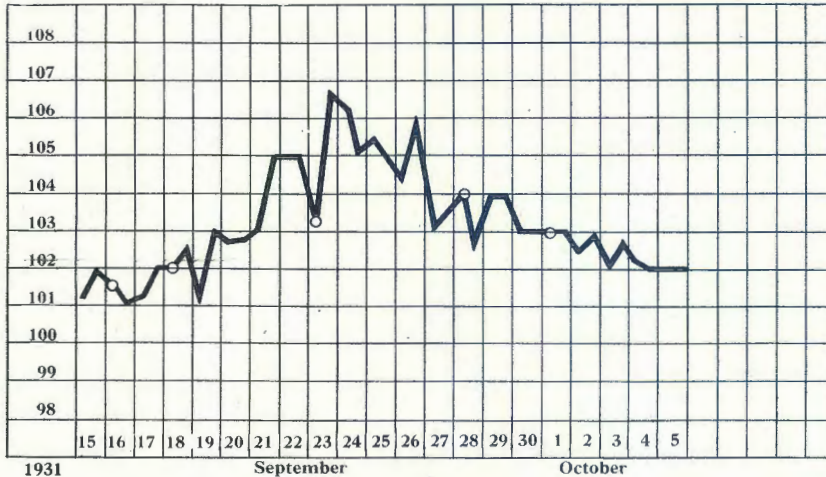
T.N.—Shows a drop coinciding with erythrocyte destruction from + 3.3-1.9 gm. per cent. within two weeks, followed by a gradual increase over the next eight weeks.

N.P.N., U.N., A.A.N., T.C.N., Sugar, and R.N.—Nothing unusual.

CASE II.

B. 3532. *Anaplasmosis (Treated and Recovered)*.

Temperature Chart II.



*History.*—B. 3532. A four-year old ox, received from Government Ranching Station, Messina, on 7/6/29. In good condition. On 1/9/31 was injected with 10 c.c. blood subcutaneously from blesbok (*Damaliscus albifrons*) 32055, which was suffering from a pure *A. marginale* infection. On the 21st day p.i., the temperature reaction set in, the first parasites being noted on the 17th day p.i. and becoming numerous from the 21st to 32nd day p.i., thereafter gradually decreasing. Morphological blood changes became noticeable on the 25th day p.i. beginning with a slight anisocytosis and running the whole gamut of changes associated with an anaplasma infection, i.e. anisocytosis, polychromasia, basophilia, normoblasts, Jolly bodies, etc. By the 43rd day p.i. only a slight anisocytosis remained. During the hyperthermic reaction the usual symptoms such as lack of appetite, dullness, staring coat and slight icterus could be observed. On the 24th day p.i. an injection was given for experimental chemotherapeutic purposes with apparently negative results, the temperature not being affected. Animal discharged 22/10/31.

TABLE 2. — *B. 3532. Anaplasmosis (Treated) Recovered.*

|                        | 1/9/31. | 4/9/31. | 9/9/31. | 16/9/31. | 18/9/31. | 23/9/31. | 28/9/31.         | 1/10/31. | 7/10/31. | 15/10/31. | 22/10/31. |
|------------------------|---------|---------|---------|----------|----------|----------|------------------|----------|----------|-----------|-----------|
| Date.....              | —       | —       | —       | —        | —        | —        | —                | —        | —        | —         | —         |
| Time.....              | N       | P.I.N.  | P.I.N.  | P.I.N.   | P.I.N.   | R        | R                | R        | N        | N         | N         |
| Temperature R.....     | —       | 14.28   | 15.42   | 15.42    | 13.87    | 16.56    | 7.80             | 9.23     | 10.61    | 13.50     | 18.57     |
| Haemoglobin gm. %..... | —       | —       | —       | —        | —        | —        | —                | —        | —        | —         | —         |
| Sugar mgm. %.....      | L       | 60.00   | —       | —        | 59.30    | 53.40    | 60.10            | 86.65    | 55.00    | 45.40     | 49.00     |
|                        | U       | 50.00   | —       | —        | 56.20    | 38.00    | —                | 70.00    | 45.00    | 31.50     | 42.00     |
| Total N. gm. %.....    | —       | 3.196   | 3.270   | 3.100    | 3.075    | 3.235    | 2.273            | 2.400    | 2.618    | 2.959     | 3.250     |
| N.P.N. mgm. %.....     | L       | 18.63   | 19.60   | 18.54    | 20.54    | 25.42    | 20.30            | 20.00    | 15.40    | 17.50     | 16.00     |
|                        | U       | 11.70   | 15.55   | 15.00    | 16.83    | 21.00    | 17.14            | 14.23    | 12.80    | 13.97     | 12.50     |
| Coag. N. mgm. N %..... | L       | 3.324   | 3.177   | 3.250    | 3.081    | 3.054    | 2.253            | 2.380    | 2.603    | 2.941     | 3.234     |
|                        | U       | 3.328   | 3.180   | 3.254    | 3.085    | 3.058    | 2.256            | 2.386    | 2.605    | 2.945     | 3.237     |
| mgm. N %.....          | L       | 3.92    | —       | —        | 5.20     | 7.10     | 10.56            | 6.90     | 4.40     | 4.83      | 4.88      |
| " U %.....             | —       | 8.19    | —       | 10.92    | 14.91    | 22.05    | 22.26            | 14.49    | 9.24     | 10.08     | 10.29     |
| mgm. N %.....          | U       | 3.50    | —       | —        | 4.88     | 7.00     | 10.17            | 6.35     | 4.19     | 4.79      | 4.88      |
| " U %.....             | —       | 7.35    | —       | 10.29    | 14.70    | 21.63    | 21.42            | 13.44    | 8.82     | 10.08     | 10.29     |
| mgm. N %.....          | L       | —       | —       | —        | 2.76     | 2.44     | 2.23             | 1.95     | 2.50     | 2.32      | 2.50      |
| " TC %.....            | —       | —       | —       | 7.46     | 6.60     | 7.20     | 6.00             | 5.14     | 6.74     | 6.26      | 6.74      |
| mgm. N %.....          | U       | —       | —       | —        | 2.23     | 1.82     | 2.23             | 1.75     | 2.10     | 2.01      | 2.23      |
| " TC %.....            | —       | —       | —       | 6.00     | 5.00     | 6.36     | 6.00             | 4.70     | 5.68     | 5.40      | 6.00      |
| mgm. N %.....          | L       | 0.53    | 0.47    | 0.50     | 0.52     | 0.53     | 0.31             | 0.27     | 0.31     | 0.41      | 0.51      |
| " UA %.....            | —       | 1.60    | 1.40    | 1.50     | 1.57     | 1.60     | 0.94             | 0.80     | 0.93     | 1.23      | 1.64      |
| mgm. N %.....          | U       | —       | —       | —        | —        | —        | —                | —        | —        | —         | —         |
| " UA %.....            | —       | —       | —       | —        | —        | —        | —                | —        | —        | —         | —         |
| Amino acid mgm N %...  | L       | 5.26    | 5.00    | 4.84     | 5.57     | 6.33     | 4.76             | 6.17     | 6.17     | 7.00      | 4.90      |
|                        | U       | 3.33    | 3.68    | 3.73     | 4.00     | 4.67     | 4.00             | 3.64     | 3.84     | 5.40      | 3.73      |
| R.N. mgm. N %.....     | L       | —       | —       | —        | 4.55*    | 4.24*    | 2.44*            | 4.71*    | 2.02*    | 2.94*     | 3.15*     |
|                        | U       | —       | —       | —        | 3.89*    | 3.89*    | 1.74*            | 2.26*    | 2.67*    | 1.77*     | 1.66*     |
| Plasma.....            | n.u.    | n.u.    | n.u.    | n.u.     | n.u.     | n.u.     | slightly icteric | n.u.     | n.u.     | n.u.      | n.u.      |

\* Indicates "Uric Acid Nitrogen."

Main Features of Analytical Data

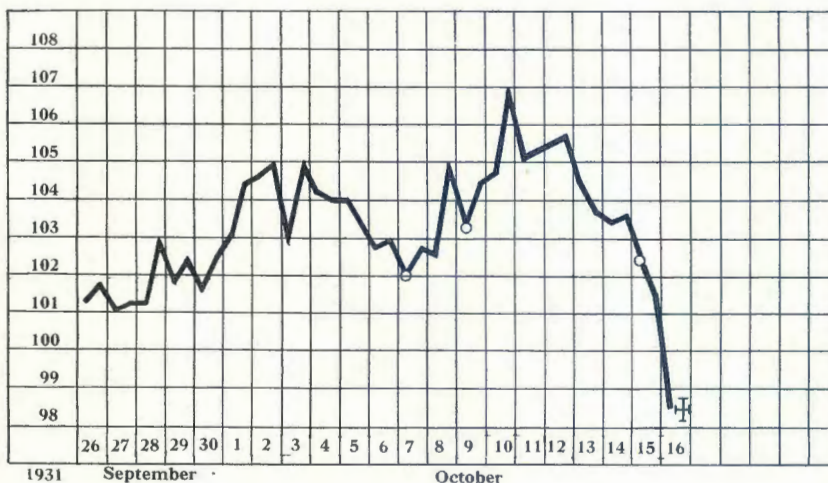
Hb.—A decrease from 15.7-8 gm. % occurs within five days followed by a steady relatively rapid return to the normal level within the succeeding three weeks.  
 N.P.N.—An increase from 20.25 mgm. N % occurs at the height of the reaction, but the normal level is reached within a few days.  
 T.C.N.—Shows an increase from 1.5-1.1 mgm. N % coinciding with the increase in N.P.N.  
 U.N.—Exhibits a decrease from 1.0-0.5 mgm. N % during the reaction; its previous level only being attained 3-4 weeks later.  
 R.N.—Shows a decrease from 1.3-3.2 gm. N % corresponding to the acute of Hb. destruction as reflected by the Hb. determination.  
 Sugar, T.C.N., U.N., R.N.—Nothing unusual.



CASE III.

B. 2888. *Anaplasmosis. Untreated—died 16/10/31.*

*Temperature Chart III.*



*History:* B. 2888. A black six-tooth ox in fair condition, bred on the Messina Government Cattle Ranch. Received here 15/2/29. Was inoculated against anthrax and blackquarter in March, 1931, and drafted into the anaplasmosis experiment 17/9/31, on which date it was injected subcutaneously with 5 c.c. blood of B. 2889 (*vide*). On the 14th day p.i. the temperature reaction set in. The animal went through a severe reaction, showing numerous anaplasmas and severe morphological blood changes; as well as marked clinical symptoms such as anorexia, dullness, icterus and severe anaemia. It died on the morning of the 30th day p.i. Post-mortem was performed within 30 minutes of death and the following pathological-anatomical changes were noted: poor condition, anaemia, generalised icterus, subepicardial petechiae, a distended gall bladder containing a thick greenish yellow bile, fatty changes and bile stasis of the liver, tumor splenis, impaction of the omasum and catarrhal enteritis—findings which substantiated the clinical diagnosis.



TABLE 3.—*B.* 2888.

| Date.....                   | 22/9/31. | 7/10/31. | 9/10/31.            | 15/10/31. |
|-----------------------------|----------|----------|---------------------|-----------|
| Time.....                   | —        | —        | —                   | —         |
| Temp. Reaction.....         | P.I.N.   | R.       | R.                  | R.        |
| Haemoglobin gm. %.....      | 12·94    | 12·01    | 13·31               | 4·33      |
| Sugar mgm. %.....           | 43·30    | 58·82    | 74·07               | 42·40     |
|                             | L        | 33·40    | 64·10               | 36·40     |
|                             | U        |          |                     |           |
| Total N. gm. %.....         | 2·913    | 2·857    | 2·548               | 1·730     |
| N.P.N. mgm. %.....          | 23·17    | 22·73    | 19·73               | 53·38     |
|                             | L        | 16·62    | 14·35               | 49·58     |
|                             | U        |          |                     |           |
| Coag. N. gm. N %.....       | 2·890    | 2·834    | 2·528               | 1·677     |
|                             | L        | 2·894    | 2·534               | 1·680     |
|                             | U        |          |                     |           |
| Urea mgm. N %.....          | —        | 7·10     | 8·10                | 31·00     |
| „ U %.....                  | —        | 15·01    | 17·01               | 65·10     |
| mgm. N %.....               | —        | 7·23     | 7·85                | 30·00     |
| „ U %.....                  | —        | 15·12    | 16·59               | 63·00     |
| Total Creatinine            |          |          |                     |           |
| mgm. N %.....               | L        | 2·54     | 2·85                | 2·10      |
| „ TC %.....                 | L        | 6·86     | 7·72                | 5·68      |
| mgm. N %.....               | U        | 2·25     | 2·50                | 2·10      |
| „ TC %.....                 | U        | 6·10     | 6·74                | 5·68      |
| Uric acid mgm. N %.....     | L        | 0·34     | 0·39                | 0·36      |
| „ UA %.....                 | L        | 1·01     | 1·18                | 1·07      |
| mgm. N %.....               | U        | 0·20     | 0·20                | 0·16      |
| „ UA %.....                 | U        | 0·60     | 0·60                | 0·48      |
| Amino acid mgm. N %.....    | L        | 7·12     | 5·60                | 6·63      |
|                             | U        | 5·51     | 4·26                | 4·91      |
| Rest Nitrogen mgm. N %..... | L        | 14·27*   | 6·79                | 2·54      |
|                             | U        | 9·66*    | 2·73                | 1·33      |
| Plasma.....                 | n.u.     | n.u.     | slightly<br>icteric | icteric   |

\* Includes "Urea Nitrogen."

† Includes "Uric Acid Nitrogen."

*Main Features of Analytical Data.*

*Hb.*—Severe drop within eight days from 12–4 gm. per cent. 24 hours before death. No haemoglobinaemia noted; bilirubinaemia distinct.

*N.P.N.*—A marked increase from about 22 mgm. to 53 mgm. N per cent. in "laked" and from 17–50 mgm. in "unlaked" filtrate.

*U.N.*—An increase from 8–31 mgm. within 6 days.

*U.A.N.*—On day before death this fraction was highest but since only one figure is involved and the increase is not beyond the normal range, a definite correlation of this finding with the condition does not seem permissible.

*A.A.N.*—A high figure was found on the last day ( $\pm$  11 mgm. N per cent.).

*R.N.*—Highest 24 hours before death.

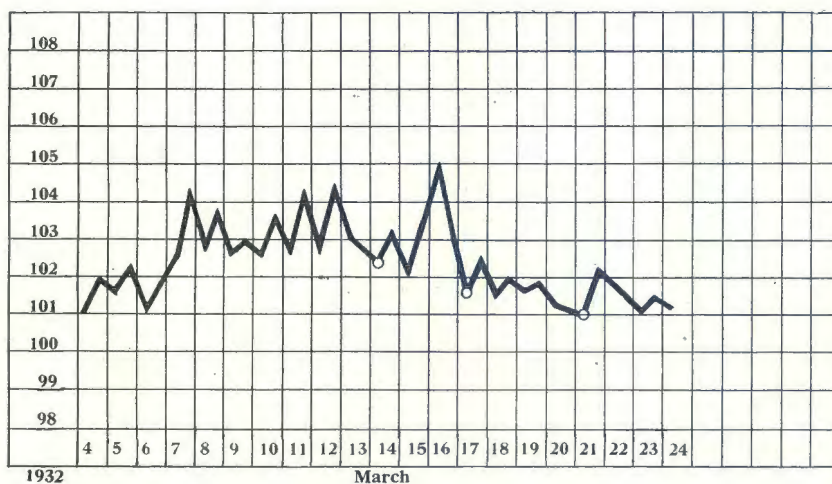
*T.N.*—Shows a drop from 2·9–1·7 gm. per cent. corresponding to the destruction of erythrocytes.

*Sugar* and *T.C.N.*—Nothing unusual.

CASE IV.

B. 3053. *Anaplasmosis. Untreated—Recovered.*

*Temperature Chart IV.*



*History.*—B. 3053. An ox, born Armoedsvlakte, 12/2/29. Was inoculated against anthrax and blackquarter 22/10/30 and tested for immunity 12 months later. Drafted into the present experiment 29/1/32, being injected subcutaneously with 5 c.c. blood from B. 2889 (*vide*) on 11/2/32. From 7/3/32 to 23/3/32 anaplasms were found in only rare numbers in blood smears, and only slight anaemic changes developed. The temperature reaction was mild. The animal was kept under observation until 4/5/32.

TABLE 4.—B. 3053. *Anaplasmosis. Untreated—Recovered.*

|                             | 8/2/32. | 11/2/32.       | 15/2/32.       | 18/2/32.       | 22/2/32.       | 25/2/32.       | 29/2/32.       | 14/3/32.       | 17/3/32.       | 21/3/32.       |
|-----------------------------|---------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Date.....                   | —       | —              | —              | —              | —              | —              | —              | —              | —              | —              |
| Time.....                   | —       | —              | —              | —              | —              | —              | —              | —              | —              | —              |
| Temp. Reaction.....         | N       | P.I.N.         | P.I.N.         | P.I.N.         | P.I.N.         | P.I.N.         | P.I.N.         | R              | R              | N              |
| Haemoglobin gm. %.....      | 18.20   | 17.51          | 15.42          | 18.57          | 16.87          | 17.18          | 15.98          | 15.19          | 16.29          | 14.28          |
| Sugar mgm. %.....           | —       | 49.00<br>39.22 | 53.48<br>28.00 | 49.00<br>33.33 | 43.43<br>35.70 | 48.10<br>48.10 | 47.20<br>41.20 | 47.40<br>38.20 | 43.86<br>36.36 | 49.70<br>40.50 |
| Total N. gm. %.....         | 3.304   | 3.346          | 2.214          | 3.430          | 3.263          | 3.290          | 3.445          | 3.130          | 3.340          | 3.032          |
| N.P.N. mgm. %.....          | L<br>U  | 22.37<br>17.07 | 25.64<br>21.06 | 21.88<br>17.20 | 22.71<br>18.10 | 21.48<br>17.05 | 22.71<br>17.23 | 17.00<br>10.00 | 21.13<br>14.10 | 20.00<br>15.90 |
| Coag. N. gm. N %.....       | L<br>U  | 3.282<br>3.287 | 3.188<br>3.193 | 3.408<br>3.413 | 3.240<br>3.245 | 3.269<br>3.273 | 3.422<br>3.428 | 3.113<br>3.120 | 3.319<br>3.326 | 3.012<br>3.016 |
| Urea mgm. N %.....          | L       | 6.17           | 4.48           | 8.70           | 9.60           | 8.20           | 8.10           | 4.60           | 5.25           | 6.63           |
| "    U %.....               | L       | 13.02          | 8.82           | 16.17          | 20.16          | 17.22          | 17.01          | 9.66           | 11.13          | 13.86          |
| mgm. N %.....               | U       | 5.52           | 4.26           | 7.80           | 9.10           | 7.00           | 8.10           | 4.40           | 5.72           | 6.23           |
| "    U %.....               | U       | 11.55          | 9.03           | 16.38          | 14.07          | 14.70          | 17.01          | 9.84           | 9.87           | 13.02          |
| Total Creatinine            |         |                |                |                |                |                |                |                |                |                |
| mgm. N %.....               | L       | 2.31           | 2.23           | 2.31           | 2.54           | 2.36           | 2.50           | 2.23           | 1.98           | 2.06           |
| "    TC %.....              | U       | 6.26           | 6.00           | 6.26           | 6.86           | 6.40           | 6.74           | 6.00           | 5.32           | 5.54           |
| mgm. N %.....               | U       | 1.91           | 1.91           | 1.88           | 2.36           | 2.00           | 2.42           | 2.10           | 1.67           | 1.84           |
| "    TC %.....              | U       | 5.14           | 5.14           | 5.06           | 6.40           | 5.40           | 6.54           | 5.68           | 4.50           | 4.96           |
| Uric acid mgm. N %.....     | L       | 0.63           | 0.61           | 0.69           | 0.57           | 0.58           | 0.54           | 0.40           | 0.49           | 0.37           |
| "    UA %.....              | U       | 1.88           | 1.83           | 2.07           | 1.70           | 1.74           | 1.62           | 1.21           | 1.48           | 1.12           |
| mgm. N %.....               | U       | 0.56           | 0.33           | 0.40           | 0.33           | 0.29           | 0.43           | 0.11           | 0.23           | 0.20           |
| "    UA %.....              | U       | 1.68           | 1.00           | 1.20           | 0.98           | 0.82           | 1.30           | 0.32           | 0.70           | 0.60           |
| Amino acid mgm. N %.....    | L<br>U  | 6.67<br>4.86   | 7.00<br>4.83   | 7.45<br>6.67   | 6.42<br>5.00   | 7.00<br>6.36   | 7.00<br>4.24   | 5.40<br>2.90   | 6.67<br>4.12   | 6.67<br>3.90   |
| Rest Nitrogen mgm. N %..... | L<br>U  | 6.59<br>4.22   | 6.96<br>3.87   | 7.19<br>4.31   | 3.65<br>2.81   | 3.34<br>1.40   | 4.36<br>2.04   | 4.37<br>0.49   | 6.74<br>3.26   | 4.27<br>3.73   |
| Plasma.....                 | n.u.    | n.u.           | n.u.           | n.u.           | n.u.           | n.u.           | n.u.           | n.u.           | n.u.           | n.u.           |

*Main Features of Analytical Data.*

*Hb.*—Tendency for a decrease during the period of reaction. Only few parasites appeared and the temperature reaction, although undoubtedly present, was only slight.  
*U.A.N.*—Shows the lowest level during the reaction  $\pm 0.60 \pm 0.45$  mgm. N %. The drop is, however, slight and not beyond the normal range encountered.  
*Sugar, N.P.N., U.N., T.C.N., A.A.N., R.N., T.N.*—Nothing unusual.  
 Although undoubtedly a positive reaction, it must be regarded as an extremely mild one and one which, under ordinary conditions would escape detection. It is possible that this animal had at some previous time passed through an anaplasmosis attack and that the present reaction represents a slight breakdown of immunity due to injection of virulent blood from B. 2889 which reacted severely.



CASE V.

B. 2308. *Anaplasmosis. Untreated—Recovered.*

*No Temperature Chart.*

*History:* B. 2308. A five-year old ox, in good condition, born on the station, 17/1/27. In December, 1931, it was immunised against anthrax and drafted on 29/1/32 into the anaplasmosis experiment, being injected subcutaneously on 11/2/32 with 5 c.c. blood from B. 2889 (*vide*). The animal was too wild to be temperatured, but frequent smear examinations revealed only a few parasites during the fifth week p.i., not associated with anaemic changes. No clinical symptoms were shown and this case must be regarded as a very mild reaction only, or even more probable an *abortive* reaction.

TABLE 5.—B. 2308. *Anaplasmosis* (No temperature—too wild.)

|                             | 8/2/32.        | 11/2/32.       | 15/2/32.       | 18/2/32.       | 22/2/32.       | 25/2/32.       | 29/2/32.       | 14/3/32.       | 17/3/32.       | 21/3/32.       |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Date.....                   |                |                |                |                |                |                |                |                |                |                |
| Time.....                   |                |                |                |                |                |                |                |                |                |                |
| Temp. Reaction.....         |                |                |                |                | Too wild.      |                |                |                |                |                |
| Huemo globin gm. %.....     | 18.57          | 17.18          | 17.16          | 19.33          | 18.22          | 17.28          | 18.20          | 20.60          | 20.18          | 13.87          |
| Sugar mgm. %.....           | —              | 42.92<br>36.76 | 37.60<br>38.80 | 51.55<br>34.01 | 40.00<br>29.60 | 51.00<br>45.80 | 50.50<br>43.90 | 40.60<br>32.50 | 43.48<br>36.90 | 46.10<br>41.15 |
| Total N. gm. %.....         | 3.480          | 3.416          | 3.444          | 3.598          | 3.648          | 3.507          | 3.578          | 3.606          | 3.662          | 3.487          |
| N.P.N. mgm. %.....          | 22.02<br>16.13 | 23.08<br>13.53 | 25.64<br>17.48 | 29.12<br>21.00 | 24.76<br>18.75 | 22.73<br>16.70 | 20.00<br>14.10 | 28.30<br>16.30 | 27.27<br>20.36 | 23.44<br>16.63 |
| Coag. N. gm. N %.....       | 3.458<br>3.464 | 3.393<br>3.399 | 3.418<br>3.427 | 3.569<br>3.577 | 3.623<br>3.629 | 3.483<br>3.490 | 3.558<br>3.564 | 3.578<br>3.590 | 3.635<br>3.642 | 3.464<br>3.410 |
| Urea mgm. N %.....          | 6.58           | 5.66           | 7.68           | 10.77          | 9.58           | 7.00           | 6.40           | 8.30           | 8.10           | 8.10           |
| "    U %.....               | 13.86          | 11.97          | 16.17          | 22.68          | 21.16          | 14.70          | 13.44          | 17.43          | 17.01          | 17.01          |
| mgm. N %.....               | 6.27           | 4.56           | 7.58           | 10.77          | 8.10           | 7.00           | 6.90           | 7.33           | 7.70           | 8.30           |
| "    U %.....               | 13.23          | 9.66           | 15.96          | 22.68          | 17.01          | 14.70          | 12.39          | 15.33          | 16.17          | 18.43          |
| Total Creatinine            |                |                |                |                |                |                |                |                |                |                |
| mgm. N %.....               | 2.31           | 2.31           | 2.13           | 2.54           | 2.96           | 2.74           | 2.50           | 2.66           | 2.29           | 2.31           |
| "    TC %.....              | 6.26           | 6.26           | 5.76           | 6.86           | 8.00           | 7.40           | 6.74           | 7.20           | 6.20           | 6.26           |
| mgm. N %.....               | 1.78           | 1.91           | 1.75           | 2.36           | 2.51           | 2.20           | 2.76           | 2.10           | 2.01           | 2.06           |
| "    TC %.....              | 4.80           | 5.14           | 4.72           | 6.40           | 7.60           | 5.86           | 7.44           | 5.68           | 5.40           | 5.54           |
| Uric acid mgm. N %.....     | 0.56           | 0.63           | 0.71           | 0.51           | 0.58           | 0.57           | 0.54           | 0.48           | 0.56           | 0.42           |
| "    UA %.....              | 1.68           | 1.88           | 2.13           | 1.54           | 1.67           | 1.70           | 1.62           | 1.44           | 1.68           | 1.21           |
| mgm. N %.....               | 0.21           | 0.25           | 0.28           | 0.32           | 0.18           | 0.20           | 0.32           | 0.30           | 0.30           | 0.31           |
| "    UA %.....              | 0.63           | 0.75           | 0.84           | 0.95           | 0.54           | 0.84           | 0.97           | 0.60           | 0.91           | 0.94           |
| Amino acid mgm. N %.....    | 6.60<br>5.64   | 6.67<br>4.00   | 7.00<br>4.67   | 7.00<br>5.38   | 8.24<br>4.83   | 6.93<br>5.83   | 7.00<br>4.00   | 7.00<br>4.24   | 7.78<br>4.38   | 7.51<br>4.38   |
| Rest Nitrogen mgm. N %..... | 5.97<br>2.23   | 7.81<br>2.87   | 8.12<br>3.20   | 8.30<br>2.17   | 3.40<br>2.83   | 4.49<br>1.39   | 3.56<br>1.12   | 10.86<br>2.43  | 9.55<br>5.97   | 5.26<br>1.58   |
| Plasma.....                 | n.u.           | n.u.           | n.u.           | n.u.           | n.u.           | n.u.           | n.u.           | n.u.           | n.u.           | n.u.           |

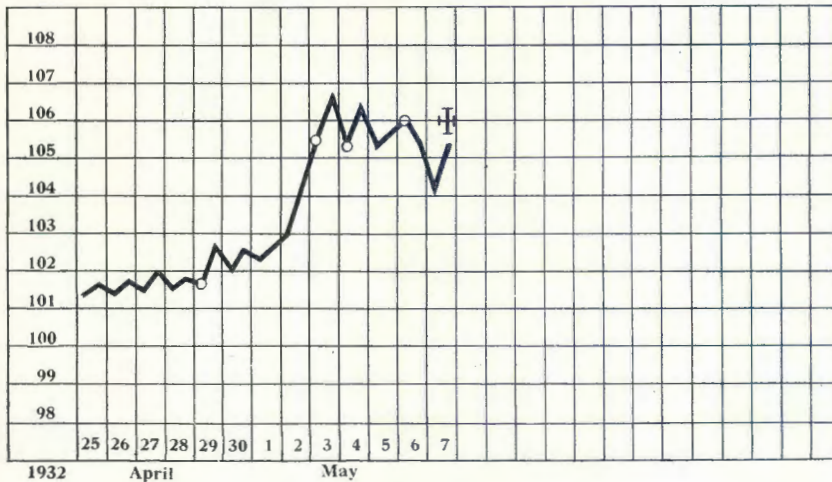
Main Features of Analytical Data.

The blood of this animal showed an *Anaplasma marginale* infection during the 4th and 5th week p.i., a temperature record being not obtainable owing to the animal's wildness. No marked anemic changes were noted. The serial analyses for each constituent revealed no distinctive features before, during or after the time the marginal points were noted, and this case therefore represents a very mild reaction only. It is of interest to note the relatively very high level of the N.P.N. fraction.

CASE VI.

B. 3132. *Anaplasmosis. Untreated—Died, 7/5/32.*

*Temperature Chart VI.*



*History*: B. 3132. A two-year old Sussex heifer, born at Onderstepoort, 24/1/30. In good condition. Inoculated in November, 1930, against anthrax and blackquarter, the immunity against anthrax being tested 12 months later. Was drafted into this experiment on 6/4/32, being injected subcutaneously with 10 c.c. blood from B. 2889 (*vide*). The first anaplasms were seen on 25/4/32, i.e. the 20th day p.i. The temperature reaction set in on 1/5/32, 26 days after inoculation. The anaplasms became very numerous and marked anaemic changes in the blood developed. The animal ceased feeding, had a tucked up appearance, a staring coat, constipation, distinct icterus and severe anaemia. Died on 7/5/32 and post-mortemed at once. The following pathological-anatomical diagnosis was made: (P.M. No. 11037 of 7/5/32). Severe anaemia, generalised icterus, fatty changes and bile pigmentation of the liver and tumor splenis, findings which support the clinical diagnosis of anaplasmosis



TABLE 6.—B. 3132. *Anaplasmosis. Untreated—Dial. 7/5/32.*

| Date.....<br>Time.....                  | 15/4/32.<br>11 a.m. | 18/4/32.       | 21/4/32.       | 28/4/32.       | 3/5/32.        | 4/5/32.        | 6/5/32.        |
|---|---------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| <i>Temp. Reaction</i> .....             | P.I.N.              | P.I.N.         | P.I.N.         | P.I.N.         | R.             | R.             | R.             |
| <i>Hb. gm. %</i> .....                  | 12.13               | 14.72          | 13.50          | 14.72          | 9.05           | 7.18           | 5.38           |
| <i>Sugar m gm. %</i> .....              | 54.35<br>40.65      | 52.91<br>39.22 | —              | 51.28<br>37.45 | 65.80<br>57.47 | 64.94<br>61.00 | 69.93<br>64.94 |
| <i>Total Nitrogen gm. %</i> .....       | 2.808               | 2.948          | 2.868          | 3.172          | 2.318          | 2.164          | 1.674          |
| <i>N.P.N. m gm. %</i> .....             | 23.20<br>17.82      | 23.08<br>16.66 | 21.43<br>15.04 | 18.75<br>13.04 | 23.08<br>18.18 | 30.00<br>25.00 | 35.70<br>30.50 |
| <i>Coag. N. gm. N %</i> .....           | 2.785<br>2.789      | 2.925<br>2.931 | 2.847<br>2.853 | 3.153<br>3.159 | 2.295<br>2.300 | 2.134<br>2.139 | 1.638<br>1.644 |
| <i>Urea m gm. N %</i> .....             | 9.00<br>18.90       | 7.16<br>15.12  | 7.81<br>16.38  | 6.40<br>13.44  | 12.16<br>25.62 | 15.67<br>33.60 | 18.30<br>38.43 |
| <i>U %</i> .....                        | 9.00                | 7.00           | 7.51           | 6.07           | 11.95          | 15.00          | 18.08          |
| <i>U %</i> .....                        | 19.90               | 14.70          | 15.75          | 13.81          | 25.20          | 31.50          | 38.00          |
| <i>Total Creatinine m gm. N %</i> ..... | 2.66<br>7.20        | 2.33<br>6.26   | 1.78<br>4.80   | 2.23<br>6.00   | 2.85<br>7.72   | 2.29<br>6.16   | 2.36<br>6.36   |
| <i>TC %</i> .....                       | 2.23                | 2.04           | 1.49           | 1.89           | 2.29           | 2.23           | 1.89           |
| <i>TC %</i> .....                       | 6.00                | 5.54           | 4.00           | 5.14           | 6.16           | 6.00           | 5.14           |
| <i>Uric Acid m gm. N %</i> .....        | 0.73<br>2.19        | 0.38<br>1.07   | 0.52<br>1.56   | 0.50<br>1.49   | 0.32<br>0.97   | 0.27<br>0.80   | 0.32<br>0.97   |
| <i>UA %</i> .....                       | 0.35                | 0.13           | 0.25           | 0.23           | 0.19           | 0.16           | 0.27           |
| <i>UA %</i> .....                       | 1.05                | 0.40           | 0.75           | 0.70           | 0.57           | 0.48           | 0.81           |
| <i>Amino acid m gm. N %</i> .....       | 6.36<br>4.24        | 7.00<br>3.90   | 6.40<br>3.68   | 6.36<br>3.68   | 5.83<br>3.18   | 5.83<br>4.38   | 5.83<br>4.52   |
| <i>Rest Nitrogen m gm. N %</i> .....    | 4.45<br>2.00        | 6.23<br>3.62   | 4.92<br>2.11   | 3.26<br>1.17   | 1.88<br>0.57   | 5.94<br>3.23   | 8.89<br>5.73   |
| <i>Plasma</i> .....                     | n.u.                | n.u.           | n.u.           | n.u.           | icteric        | —              | —              |

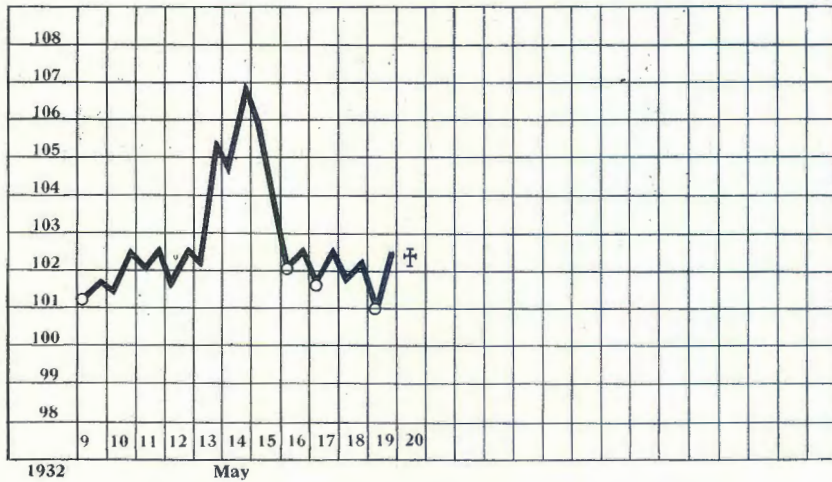
*Main Features of Analytical Data.*

*Hb.*—Shows a marked decrease from 14.5-3 gm. % 24 hours prior to death; this drop supervening within eight days. No Jaemoglobinaemia, but bilirubinaemia was noticeable.  
*Sugar.*—Showed a slight tendency towards an increase in both filtrates.  
*N.P.N.*—Increased from ± 20-36 m gm. N % and from ± 17-30.50 m gm. N % in “laked” and “unlaked” filtrates respectively.  
*U.N.*—A definite increase from 8-18 m gm. N % is noted.  
*U.A.N.*—Shows a tendency to decrease in both filtrates.  
*R.N.*—An increase is observed shortly before death.  
*T.N.*—Drops from 2.8-1.7 gm. N % paralleling the decline in Hb. content.  
*T.C.N.*, *A.A.N.*—Nothing unusual.

CASE VII.

B. 2269. *Anaplasmosis*. Treated—Died, 20/5/32.

Temperature Chart VII.



*History*: B. 2269. A five-year-old cross-bred ox in fair condition. Immunised against anthrax, 4/12/31 and drafted into anaplasmosis experiment 6/4/32, on which date it was injected with 10 c.c. blood from B. 3053 (*vide*). Anaplasms were first noted the 30th day p.i. and the temperature rose on the 38th day p.i. The parasites became numerous and anaemic blood changes developed. The animal ceased feeding, developed constipation, salivation and icterus, and died on the 44th day p.i. Received an injection on the 14th and 19th May.

At post-mortem (P.M. No. 11128 of 20/5/32) the following was found: generalised icterus, anaemia, cholaemia, slight oedema and emphysema of the lungs, acute tumor splenis, tumor hepatitis and pigmentation. A haemoglobinuria was also noted (sequel therapeutic injection?).

TABLE 7.—*B. 2269. Anaplasmosis. Treated—Died, 7.30 a.m. on 20/5/32.*

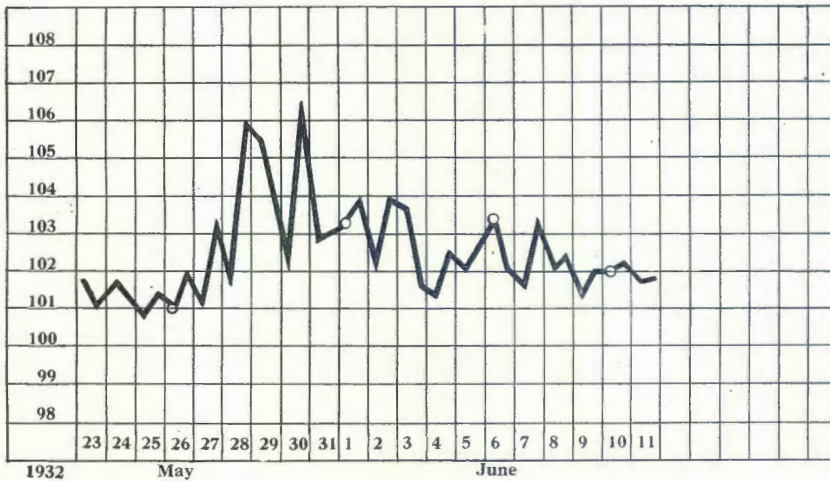
| Date.....                   | 12/4/32.<br>11 a.m. | 14/4/32.<br>12 a.m. | 18/4/32.       | 21/4/32.       | 28/4/32.       | 4/5/32.        | 9/5/32.        | 16/5/32.       | 17/5/32.       | 19/5/32.       |
|-----------------------------|---------------------|---------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Temp. Reaction.....         | P.I.N.              | P.I.N.              | P.I.N.         | P.I.N.         | P.I.N.         | P.I.N.         | P.I.N.         | P.I.N.         | P.I.N.         | R.             |
| Haemoglobin gm. %.....      | 14.49               | 18.53               | 19.73          | 18.57          | 23.18          | 18.20          | 21.18          | 15.42          | 12.42          | 6.44           |
| Sugar mgm. %.....           | L<br>66.00          | 78.74<br>66.00      | 66.30<br>38.33 | 51.55<br>35.00 | 47.17<br>36.00 | 43.17<br>31.45 | 53.76<br>42.37 | 65.52<br>55.87 | 61.35<br>51.80 | 30.58<br>26.04 |
| Total Nitrogen gm. %.....   | 3.242               | 3.564               | 3.548          | 3.648          | 3.774          | 3.690          | 3.522          | 2.948          | 2.780          | 2.024          |
| N.P.N. mgm. %.....          | L<br>U              | 25.00<br>18.75      | 33.33<br>25.00 | 23.08<br>15.43 | 22.06<br>18.73 | 21.43<br>15.80 | 24.50<br>18.46 | 30.94<br>23.10 | 28.60<br>21.00 | 51.38<br>45.12 |
| Coag. N. gm. N %.....       | L<br>U              | 3.217<br>3.221      | 3.531<br>3.539 | 3.625<br>3.633 | 3.752<br>3.755 | 3.669<br>3.674 | 3.498<br>3.504 | 2.917<br>2.925 | 2.751<br>2.759 | 1.973<br>1.979 |
| Urea mgm. N %.....          | L                   | 11.50               | 11.50          | 9.25           | 8.75           | 6.69           | 9.52           | 15.62          | 10.24          | 19.20          |
| " U %.....                  | U                   | 24.15               | 24.15          | 19.53          | 18.48          | 14.07          | 20.00          | 32.76          | 21.72          | 40.32          |
| " U %.....                  | U                   | 11.12               | 10.90          | 8.52           | 9.00           | 6.02           | 8.52           | 14.62          | 10.00          | 18.61          |
| " U %.....                  | U                   | 23.31               | 22.89          | 17.85          | 18.90          | 12.60          | 17.85          | 30.66          | 21.00          | 39.06          |
| Total Creatinine            |                     |                     |                |                |                |                |                |                |                |                |
| mgm. N %.....               | L                   | 2.23                | 2.31           | 2.23           | 1.97           | 2.11           | 2.11           | 2.35           | 3.08           | 2.42           |
| " TC %.....                 | U                   | 6.00                | 6.26           | 6.00           | 5.32           | 5.68           | 5.68           | 6.36           | 8.30           | 6.54           |
| mgm. N %.....               | U                   | 2.06                | 2.96           | 1.67           | 1.82           | 1.67           | 1.75           | 1.82           | 2.27           | 2.17           |
| " TC %.....                 | U                   | 5.54                | 8.00           | 5.14           | 4.50           | 4.90           | 4.70           | 4.90           | 6.16           | 5.84           |
| Uric acid mgm. N %.....     | L                   | 0.30                | 0.42           | 0.33           | 0.71           | 0.45           | 0.40           | 0.37           | 0.37           | 0.47           |
| " UA %.....                 | U                   | 0.90                | 1.25           | 1.00           | 2.14           | 1.36           | 1.20           | 1.10           | 1.10           | 1.41           |
| mgm. N %.....               | U                   | 0.21                | 0.15           | 0.20           | 0.50           | 0.32           | 0.22           | 0.71           | 0.19           | 0.36           |
| " UA %.....                 | U                   | 0.64                | 0.45           | 0.59           | 1.50           | 0.96           | 0.67           | 0.52           | 0.57           | 1.09           |
| Amino acid mgm. N %.....    | L<br>U              | 7.78<br>4.24        | 6.67<br>4.12   | 7.87<br>3.50   | 7.57<br>3.40   | 7.00<br>4.12   | 8.24<br>3.90   | 8.24<br>4.03   | 8.24<br>5.11   | 14.58<br>11.66 |
| Rest Nitrogen mgm. N %..... | L<br>U              | 3.19<br>1.12        | 12.07<br>6.87  | 7.22<br>2.02   | 3.58<br>1.34   | 3.63<br>3.47   | 4.69<br>3.29   | 4.36<br>2.46   | 6.67<br>3.44   | 14.71<br>12.38 |
| Plasma.....                 | n.u.                | n.u.                | n.u.           | n.u.           | n.u.           | n.u.           | n.u.           | icteric        | icteric        | icteric        |

*Main Features of Analytical Data.*

*Hb*—A marked decrease from  $\pm 20$ -6 gm. % is noted. Towards death the drop is very severe, the *Hb.* level dropping within 48 hours from 12.6 gm. % 24 hours before death.  
*N.P.N.*—Shows a sharp rise in both filtrates from  $\pm 24$ -57 mgm. N % and from  $\pm 18$ -45 mgm. N % in "laked" and "unlaked" filtrates respectively.  
*U.N.*—Rises from  $\pm 8$ -19 mgm. N % in both filtrates, the increase being particularly striking 24 hours before death.  
*A.A.N.*—Increases considerably in both filtrates shortly before death.  
*R.N.*—Here also an increase can be recorded from  $\pm 5$ -16 mgm. N % and from  $\pm 4$ -12 mgm. N % in "laked" and "unlaked."  
*Sugar and T.C.N.*—Nothing unusual.



## CASE VIII.

B. 3198. *Anaplasmosis. Treated—Recovered.**Temperature Chart VIII.*

*History:* B. 3198. A two-year-old heifer (born at Onderstepoort on 5/3/30), in good condition. Was inoculated against anthrax and blackquarter in November, 1930, the anthrax immunity being tested 12 months later. It was then drafted into the anaplasmosis experiment on 6/5/32, receiving 10 c.c. blood from bovine 3132 (*vide*) subcutaneously. Frequent smear examinations were made. On 21/5/32, i.e. 15 days p.i., the first *Anaplasma marginale* appeared. Before the temperature rose, the parasites were fairly frequent, and anisocytosis was noted, and by 1/6/32 marked blood changes, e.g. basophilia, polychromasia, normoblasts had developed. The parasites in rare numbers continued to be present for 5-6 weeks, but the blood changes showed a continuous improvement, so that by 7/7/32 only a slight anisocytosis remained. During the reaction the animal ceased feeding, was dull, showed icterus and passed only small amounts of rather firm faeces. A chemotherapeutic injection was given during the main reaction (30/5/32).

TABLE 8.—B. 3198. *Anaplasmosis. Treated—Recovered.*

| Date.....                | 9/5/32.                  | 13/5/32.       | 17/5/32.       | 26/5/32.       | 1/6/32.        | 6/6/32.        | 10/6/32.       | 14/6/32.       | 20/6/32.<br>10.30 a.m. | 24/6/32.       |
|--------------------------|--------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|------------------------|----------------|
| Time.....                |                          |                |                |                |                |                |                |                |                        |                |
| Temp. Reaction.....      | P.I.N.                   | P.I.N.         | P.I.N.         | P.I.N.         | R.             | R.             | R.             | R.             | N.                     | N.             |
| Haemoglobin gm. %.....   | 15.42                    | 14.95          | 13.87          | 11.53          | 4.91           | 4.45           | 5.59           | 7.66           | 10.35                  | 10.99          |
| Sugar mgm. %.....        | L<br>59.88<br>U<br>51.28 | 60.61<br>46.08 | 61.35<br>53.19 | 61.35<br>52.91 | 71.94<br>66.67 | 80.00<br>64.10 | —              | —              | —                      | 52.86<br>45.66 |
| Total N. gm. N %.....    | 3.032                    | 2.941          | 2.814          | 2.612          | 1.730          | 1.611          | 1.884          | 2.206          | 2.360                  | 2.339          |
| N.P.N. mgm. %.....       | L<br>25.00<br>U<br>17.27 | 21.43<br>15.04 | 17.65<br>12.00 | 13.63<br>10.00 | 27.71<br>23.08 | 18.75<br>15.00 | 14.85<br>10.71 | 15.39<br>9.75  | 13.40<br>9.40          | 13.04<br>9.84  |
| Coag. N. gm. N %.....    | L<br>3.007<br>U<br>3.015 | 2.920<br>2.926 | 2.796<br>2.802 | 2.598<br>2.602 | 1.702<br>1.707 | 1.592<br>1.596 | 1.869<br>1.873 | 2.191<br>2.196 | 2.347<br>2.351         | 2.326<br>2.329 |
| Urea mgm. N %.....       | L<br>9.00                | 8.09           | 4.63           | 3.50           | 15.26          | 7.85           | 4.48           | 3.41           | 3.65                   | 3.08           |
| "    U %.....            | 18.90                    | 17.01          | 9.66           | 7.35           | 32.13          | 16.59          | 9.45           | 7.14           | 7.77                   | 6.51           |
| "    N %.....            | 8.09                     | 7.97           | 4.45           | 3.30           | 15.00          | 7.66           | 4.41           | 3.20           | 3.76                   | 3.08           |
| "    U %.....            | 17.01                    | 16.80          | 9.45           | 6.93           | 31.50          | 16.17          | 9.24           | 6.72           | 7.98                   | 6.51           |
| Total Creatinine         |                          |                |                |                |                |                |                |                |                        |                |
| mgm. N %.....            | L<br>2.23                | 1.87           | 2.66           | 2.01           | 2.35           | 1.71           | —              | —              | —                      | 2.04           |
| "    TC %.....           | 6.00                     | 5.02           | 7.20           | 5.40           | 6.36           | 4.60           | —              | —              | —                      | 5.50           |
| mgm. N %.....            | U<br>1.98                | 1.61           | 2.17           | 1.64           | 1.67           | 1.54           | —              | —              | —                      | 1.75           |
| "    TC %.....           | 5.32                     | 4.32           | 5.84           | 4.40           | 4.50           | 4.16           | —              | —              | —                      | 4.70           |
| Uric acid mgm. N %.....  | L<br>0.44                | 0.42           | 0.46           | 0.39           | 0.32           | 0.26           | —              | —              | —                      | 0.36           |
| "    UA %.....           | 1.31                     | 1.25           | 1.38           | 1.18           | 0.97           | 0.78           | —              | —              | —                      | 1.07           |
| mgm. N %.....            | U<br>0.17                | 0.18           | 0.19           | 0.18           | 0.22           | 0.20           | —              | —              | —                      | 0.18           |
| "    UA %.....           | 0.50                     | 0.55           | 0.56           | 0.53           | 0.67           | 0.60           | —              | —              | —                      | 0.54           |
| Amino acid mgm. N %..... | L<br>6.86                | 6.28           | 6.36           | 5.00           | 6.14           | 4.49           | 4.38           | 5.17           | 4.02                   | 4.67           |
| "    U %.....            | 3.50                     | 3.50           | 3.33           | 3.11           | 3.68           | 2.92           | 2.72           | 2.92           | 3.15                   | 2.72           |
| Rest Nitrogen gm. %..... | L<br>6.47                | 4.77           | 3.54           | 2.73           | 3.64           | 4.34           | 5.99*          | 6.78*          | 5.73*                  | 2.89           |
| "    U %.....            | 3.53                     | 1.78           | 1.86           | 1.77           | 2.51           | 2.68           | 3.58*          | 3.63*          | 2.59*                  | 2.11           |

\* Includes "Total Creatinine Nitrogen" and "Uric Acid Nitrogen."

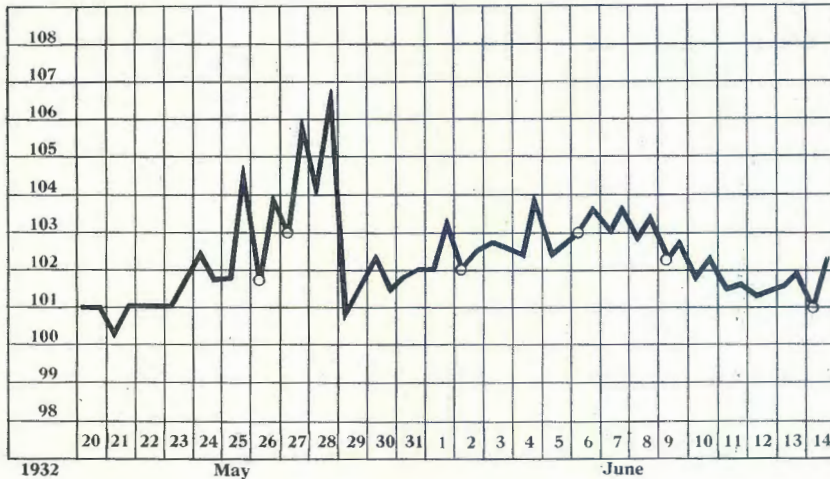
*Main Features of Analytical Data.*

*Hb.*—This shows a decrease commencing before the actual temperature reaction set in, the Hb. falling from 15.4-45 gm. % over a period of about three weeks, an improvement setting in as soon as the temperature has reached normal.  
*Sugar.*—Shows a tendency towards a slight increase.  
*N.P.N. and U.N.*—Both these fractions increase during the actual temperature reaction, dropping to the normal level before even the hyperthermia has ended.  
*U.A.N.*—Shows a decrease during the reaction, the return to normal being prolonged for a considerable period.  
*T.C.N.*—A decrease from 3.0-1.6 gm. % can be recorded during the course of the condition, recovery being somewhat retarded.  
*T.C.N. and A.A.N.*—Nothing unusual.

CASE IX.

B. 3126. *Anaplasmosis. Treated—Recovered.*

Temperature Chart IX.



*History:* B. 3126. A two year old tolly in good condition (born Armoeds-vlakte, 17/1/30). Immunised against blackquarter and anthrax in November, 1930, anthrax immunity being tested 12 months later. Placed into anaplasmosis experiment on 6/5/32, being injected subcutaneously with 10 c.c. blood from B. 3132 (*vide*). Fifteen days later the first anaplasms appeared in the blood, the temperature rising on the 20th day p.i. The anaplasms became increasingly numerous and severe anaemic changes including anisocytosis, polychromasia, basophilia, and Jolly bodies developed. The animal showed clinically loss of appetite, dullness, slight icterus and constipation. Chemotherapeutic treatment was undertaken on the 20th and 30th May, and the 2nd June with apparently favourable results, the parasites becoming markedly decreased in number. The anaemic changes in the blood gradually disappeared, only a slight anisocytosis persisting for another few weeks. The animal made an uneventful recovery and was discharged on 17/7/32.



TABLE 9.—B. 3126. *Anaplasmosis. Treated—Recovered.*

| Date.....                   | 9/5/32. | 13/5/32. | 17/5/32. | 26/5/32. | 27/5/32. | 2/6/32.<br>7.15 a.m. | 6/6/32. | 9/6/32. | 14/6/32. | 20/6/32. | 24/6/32. | 1/7/32. | 8/7/32. | 14/7/32. |
|-----------------------------|---------|----------|----------|----------|----------|----------------------|---------|---------|----------|----------|----------|---------|---------|----------|
| Time.....                   | —       | —        | —        | —        | —        | —                    | —       | —       | —        | —        | —        | —       | —       | —        |
| Temp. Reaction.....         | P.I.N.  | P.I.N.   | P.I.N.   | R.       | R.       | R.                   | R.      | R.      | R.       | N.       | N.       | N.      | N.      | N.       |
| Haemoglobin gm. %.....      | 20.60   | 20.18    | 18.20    | 13.12    | 11.39    | 4.91                 | 4.74    | 5.32    | 8.14     | 11.39    | 12.42    | 14.28   | 14.95   | 15.19    |
| Sugar mgn. %.....           | L       | 52.63    | 49.02    | 58.82    | 59.17    | 75.19                | 86.21   | 79.36   | —        | —        | 49.02    | 49.75   | 54.95   | 53.48    |
|                             | U       | 44.44    | 41.32    | 46.30    | 53.48    | 68.97                | 74.63   | 74.63   | —        | —        | 43.86    | 46.87   | 42.37   | 43.10    |
| Total N. gm. N %.....       | 3.550   | 3.480    | 3.382    | 2.990    | 2.675    | 1.820                | 1.814   | 1.842   | 2.248    | 2.717    | 2.892    | 3.011   | 2.976   | 3.193    |
| N.P.N. mgn. %.....          | L       | 27.27    | 22.22    | 21.43    | 20.00    | 20.00                | 19.54   | 17.88   | 15.79    | 15.25    | 15.85    | 14.92   | 12.40   | 15.75    |
|                             | U       | 17.15    | 15.72    | 15.28    | 15.08    | 17.97                | 14.28   | 12.50   | 10.00    | 8.57     | 12.20    | 11.77   | 10.00   | 10.10    |
| Coag. N. gm. N %.....       | L       | 3.523    | 3.458    | 3.361    | 2.970    | 2.655                | 1.794   | 1.824   | 2.232    | 2.702    | 2.876    | 2.996   | 2.964   | 3.176    |
|                             | U       | 3.533    | 3.564    | 3.367    | 2.975    | 2.660                | 1.802   | 1.830   | 2.238    | 2.798    | 2.880    | 2.999   | 2.966   | 3.183    |
| Urea mgn. N %.....          | L       | 9.00     | 8.66     | 8.05     | 8.17     | 8.52                 | 9.00    | 7.47    | 4.68     | 3.00     | 5.78     | 4.26    | 3.76    | 3.88     |
| " U %.....                  | L       | 18.90    | 18.17    | 17.00    | 17.22    | 17.85                | 18.90   | 15.33   | 9.87     | 6.30     | 12.18    | 9.03    | 8.00    | 8.19     |
| mgn. N %.....               | U       | 8.30     | 8.09     | 7.51     | 7.51     | 8.52                 | 9.52    | 5.92    | 4.13     | 2.92     | 5.49     | 4.06    | 3.65    | 3.65     |
| " U %.....                  | U       | 17.43    | 17.00    | 15.75    | 15.75    | 17.85                | 20.00   | 15.00   | 8.61     | 6.09     | 11.55    | 8.71    | 7.56    | 7.56     |
| Total Creatinine            |         |          |          |          |          |                      |         |         |          |          |          |         |         |          |
| mgn. N %.....               | L       | 2.04     | 2.04     | 2.96     | 2.35     | 2.42                 | 2.57    | 2.23    | —        | —        | 2.50     | 2.34    | 2.17    | 2.23     |
| " TC %.....                 | L       | 5.50     | 5.50     | 8.00     | 6.36     | 6.54                 | 6.96    | 6.00    | —        | —        | 6.74     | 6.36    | 5.84    | 6.00     |
| mgn. N %.....               | U       | 1.91     | 1.61     | 2.57     | 1.82     | 2.10                 | 2.01    | 1.49    | —        | —        | 1.98     | 1.75    | 1.71    | 2.01     |
| " TC %.....                 | U       | 5.14     | 4.32     | 6.96     | 4.90     | 5.68                 | 5.40    | 4.00    | —        | —        | 5.32     | 4.70    | 4.60    | 5.40     |
| Uric acid mgn. N %.....     | L       | 0.33     | 0.37     | 0.38     | 0.33     | 0.29                 | 0.25    | 0.30    | —        | —        | 0.29     | —       | 0.31    | 0.38     |
| " UA %.....                 | L       | 0.99     | 1.02     | 1.14     | 0.98     | 0.87                 | 0.74    | 0.89    | —        | —        | 0.87     | —       | 0.94    | 1.07     |
| mgn. N %.....               | U       | 0.13     | 0.16     | 0.14     | 0.16     | 0.13                 | 0.15    | 0.28    | —        | —        | 0.16     | —       | 0.18    | 0.18     |
| " UA %.....                 | U       | 0.40     | 0.48     | 0.42     | 0.47     | 0.40                 | 0.44    | 0.84    | —        | —        | 0.48     | —       | 0.55    | 0.53     |
| Amino acid mgn. N %.....    | L       | 7.78     | 6.80     | 6.67     | 5.83     | 5.69                 | 5.60    | 4.93    | 4.61     | 4.67     | 4.52     | 4.93    | 4.58    | 4.67     |
|                             | U       | 3.68     | 3.68     | 3.18     | 3.68     | 3.18                 | 3.50    | 2.72    | 2.60     | 2.72     | 2.92     | 3.50    | 2.50    | 2.72     |
| Rest Nitrogen mgn. N %..... | L       | 8.12     | 4.35     | 3.37     | 3.32     | 3.08                 | 2.58    | 4.45    | 6.50*    | 7.58*    | 2.76     | 3.39†   | 3.58    | 4.54     |
|                             | U       | 3.13     | 2.18     | 1.88     | 1.91     | 1.07                 | 2.79    | 2.09    | 3.27*    | 2.93*    | 1.65     | 2.46†   | 1.96    | 1.54     |
| Plasma.....                 | n.u.    | n.u.     | n.u.     | icteric  | icteric  | icteric              | icteric | n.u.    | n.u.     | n.u.     | n.u.     | n.u.    | n.u.    | n.u.     |

\* Includes "Total Creatinine Nitrogen" and "Uric Acid Nitrogen." † Includes "Uric Acid Nitrogen."

Main Features of Analytical Data.

Hb.—Drops from 20-4.7 gm. % over a period of three weeks, and returns to the normal level setting in before the temperature has dropped. Haemoglobinemia absent, with anaemia present.  
 Sugar.—Tendency to increase.  
 N.P.N. and U.N.—An interesting feature of these two N. fractions in this case is that no significant changes in amount occur, in spite of the relatively heavy drop in Hb. contents, and severe morphological blood changes.  
 T.C.N., U.N.N., A.A.N., and R.N.—Nothing unusual.  
 T.N.—Decreases from 3.5-1.8 gm. N %, the lowest level being reached when the Hb. level is at its lowest.



## (d) THE NORMAL RANGE OF VALUES OBTAINED FOR Hb., SUGAR, N.P.N., U.N., T.C.N., U.A.N., A.A.N., R.N. AND T.N. IN BOVINE BLOOD.

Before it is possible to determine what represents abnormal concentrations of any particular constituent, the normal variations, preferably determined under the same environmental conditions, must be known. Fragmentary researches in this connection exist for most or all of the domestic animals but no complete range of data comprising a relatively large number of animals of different breeds and ages of one species on a fixed diet and representing analyses taken over a period of 12-18 months to note seasonal variations, effect of pregnancy, diet, etc., exists. A further complication arises out of the fact that with different methods different results are obtained, the variations in some cases being considerable, and comparisons of results obtained by different workers becomes difficult or impossible. Analyses taken over short periods, or worse still, representing one single analysis, are of little value, since the concentrations of various constituents may vary considerably—even on a fixed diet—during the course of a year, due to ageing of the animal and seasonal variation. This is well brought out in a series of analyses made on sheep blood by Hamersma (1933) over a period of about twelve months. The same tendencies can be observed in some of the serial analytical figures submitted here, particularly in those *bovines* for which the data collected extends over ten weeks.

The "normal" figures utilised here represent 50-60 analyses of "normal" bloods from animals of various breeds and ages and made during different times of the year. Some of the data are from animals not tabulated here, but which were intended for infection especially in connection with redwater experiments, but which proved refractory. In each case the minimum and maximum range is given; the "average" obtained by adding all the normal data and dividing by the respective number of analyses, and tables to indicate the grouping of data. The percentage variation of constituents in the "laked" and "unlaked" filtrates respectively, is also given, together with the "average" percentage variation. The latter has primarily been given for the purpose of obtaining "factors" which would enable figures obtained from "laked" filtrate analyses being recalculated in terms of "unlaked" filtrates and *vice versa*, and thus form some basis for comparison of analytical data gathered by different research workers using either filtrate.

*Haemoglobin.*

Minimum-maximum range: 12.1-23.2 gm. Hb. %.

"Average" Hb. content: 17.1 gm. Hb. %.

*Grouping according to distribution.*

| <i>gm. Hb. %.</i> | <i>Occurrence.</i> | <i>gm. Hb. %.</i> | <i>Occurrence.</i> |
|-------------------|--------------------|-------------------|--------------------|
| 11-12             | 0                  | 17-18             | 7                  |
| 12-13             | 2                  | 18-19             | 9                  |
| 13-14             | 2                  | 19-20             | 2                  |
| 14-15             | 7                  | 20-21             | 4                  |
| 15-16             | 5                  | 21-22             | 1                  |
| 16-17             | 3                  | 22-23             | 1                  |

*Sugar.*

Minimum-maximum variation "laked" 37.6-95 mgm. %.

"Average laked," 60 mgm. %.

Minimum-maximum variation "unlaked," 31.5-82.6 mgm. %.

Average "unlaked," 47.8 mgm. %.

Percentage difference between "laked" and "unlaked" filtrates range from 4-27 % with an average variation of 20 %, the "laked" being the higher.

*Grouping according to distribution.*

| <i>"Laked."</i> |                    | <i>"Unlaked."</i> |                    |
|-----------------|--------------------|-------------------|--------------------|
| <i>mgm. %.</i>  | <i>Occurrence.</i> | <i>mgm. %.</i>    | <i>Occurrence.</i> |
| 35-45           | 7                  | 30-40             | 15                 |
| 45-55           | 17                 | 40-50             | 12                 |
| 55-65           | 7                  | 50-60             | 6                  |
| 65-75           | 4                  | 60-70             | 5                  |
| 75-85           | 2                  | 70-80             | 1                  |
| 85-95           | 3                  | 80-90             | 1                  |

*Total Nitrogen.*

Minimum-maximum variation, 2.5-3.5 gm. N %.

Average, 3 gm. N %.

*Grouping according to distribution.*

| <i>gm. N %.</i> | <i>Occurrence.</i> |
|-----------------|--------------------|
| 2.5-2.7         | 8                  |
| 2.7-2.9         | 7                  |
| 2.9-3.1         | 4                  |
| 3.1-3.3         | 7                  |
| 3.3-3.5         | 6                  |

*Non-Protein Nitrogen.*

Minimum-maximum variation "laked," 12.5-25 mgm. N %.

Average "laked," 18.2 mgm. N %.

Minimum-maximum variation "unlaked," 9.4-18.7 mgm. N %.

Average "unlaked," 13.5 mgm. N %.

Percentage differences between "laked" and "unlaked" filtrates range from 12.7-36.2 % with an average of 20.3 %; the "laked" concentration being always higher.

*Grouping according to distribution.*

| <i>"Laked."</i>  |                    | <i>"Unlaked."</i> |                    |
|------------------|--------------------|-------------------|--------------------|
| <i>mgm. N %.</i> | <i>Occurrence.</i> | <i>mgm. N %.</i>  | <i>Occurrence.</i> |
| 12-14            | 4                  | Under 11          | 0                  |
| 14-16            | 6                  | 9-11              | 9                  |
| 16-18            | 9                  | 11-13             | 9                  |
| 18-20            | 11                 | 13-15             | 10                 |
| 20-22            | 4                  | 15-17             | 7                  |
| 22-24            | 4                  | 17-19             | 5                  |
| 24-26            | 2                  | Over 19           | 0                  |

*Urea Nitrogen.*

Owing to the only small difference between the "laked" and "unlaked" U.N. concentration no differentiation is made between the two filtrates. The "unlaked" U.N. is in the vast majority of cases slightly lower.

Minimum-maximum variation, 3.0-9.0 mgm. N %.

Average, 5.7 mgm. N %.

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*Grouping according to distribution.*

| <i>mgm. N %.</i> | <i>Occurrence.</i> | <i>mgm. N %.</i> | <i>Occurrence.</i> |
|------------------|--------------------|------------------|--------------------|
| 3-4              | 12                 | 6-7              | 16                 |
| 4-5              | 12                 | 7-8              | 3                  |
| 5-6              | 12                 | 8-9              | 5                  |

*Total Creatinine Nitrogen.*

Minimum-maximum variation "laked," 1.9-2.9 mgm. N %.

Average "laked," 2.3 mgm. N %.

Minimum-maximum variation "unlaked," 1.4-2.6 mgm. N %.

Average "unlaked," 1.9 mgm. N %.

Percentage difference between "laked" and "unlaked" filtrates vary from 3-30 % with an average variation of 14 %, the "laked" being the higher.

*Grouping according to distribution.*

| <i>" Laked.</i>  |                    | <i>" Unlaked."</i> |                    |
|------------------|--------------------|--------------------|--------------------|
| <i>mgm. N %.</i> | <i>Occurrence.</i> | <i>mgm. N %.</i>   | <i>Occurrence.</i> |
| 1.9-2.1          | 9                  | 1.4-1.6            | 2                  |
| 2.1-2.3          | 13                 | 1.6-1.8            | 10                 |
| 2.3-2.5          | 3                  | 1.8-2.0            | 8                  |
| 2.5-2.7          | 3                  | 2.0-2.2            | 3                  |
| 2.7-2.9          | 2                  | 2.2-2.4            | 5                  |
| —                | —                  | 2.4-2.6            | 2                  |

*Uric Acid Nitrogen.*

Minimum-maximum variation "laked," .33-.73 mgm. N %.

Average "laked," .46 mgm. N %.

Minimum-Maximum variation "unlaked," .14-.56 mgm. N %.

Average "unlaked," .23 mgm. N %.

Percentage difference between "laked" and "unlaked" filtrates vary from 9-63 % with an average of 50 %, the "laked" being the higher.

*Grouping according to distribution.*

| <i>" Laked."</i> |                    | <i>" Unlaked."</i> |                    |
|------------------|--------------------|--------------------|--------------------|
| <i>mgm. N %.</i> | <i>Occurrence.</i> | <i>mgm. N %.</i>   | <i>Occurrence.</i> |
| .33-.43          | 16                 | .14-.24            | 22                 |
| .43-.53          | 6                  | .24-.34            | 5                  |
| .53-.63          | 5                  | .34-.44            | 2                  |
| .63-.73          | 3                  | .44-.56            | 1                  |

*Amino Acid Nitrogen.*

Minimum-maximum variation "laked," 4.3-8.4 mgm. N %.

Average "laked," 5.8 mgm. N %.

Minimum-maximum variation "unlaked," 2.1-5.6 mgm. N %.

Average "unlaked," 3.8 mgm. N %.

Percentage differences between "laked" and "unlaked" filtrates vary from 14-50 % with an average of 34.6 %, the "laked" being the higher.



## Grouping according to distribution.

| "Laked."  |             | "Unlaked." |             |
|-----------|-------------|------------|-------------|
| mgm. N %. | Occurrence. | mgm. N %.  | Occurrence. |
| 4.3-5.4   | 15          | 2.1-3.1    | 10          |
| 5.4-6.4   | 5           | 3.1-4.1    | 10          |
| 6.5-7.4   | 8           | 4.1-5.1    | 4           |
| 7.4-8.4   | 2           | 5.1-6.1    | 6           |

## Rest Nitrogen.

Minimum-maximum variation "laked," 2.0-6.6 mgm. N %.

Average "laked," 4.0 mgm. N %.

Minimum-maximum variation "unlaked," 1.4-4.2 mgm. N %.

Average "unlaked," 2.1 mgm. N %.

Percentage differences between "laked" and "unlaked" filtrates vary from 0-72 % with an average of 44 %, the "laked" being always the higher.

## Grouping according to distribution.

| "Laked."  |             | "Unlaked." |             |
|-----------|-------------|------------|-------------|
| mgm. N %. | Occurrence. | mgm. N %.  | Occurrence. |
| 2.0-2.5   | 2           | 1.4-2.0    | 11          |
| 2.5-3.0   | 4           | 2.0-2.5    | 6           |
| 3.0-4.5   | 7           | 2.5-3.0    | 1           |
| 4.5-5.5   | 5           | 3.0-3.5    | 2           |
| 5.5-6.0   | 0           | 3.5-4.0    | 0           |
| 6.0-6.6   | 2           | 4.0-4.5    | 1           |

## (e) GENERAL SUMMARY OF PATHOLOGICAL FINDINGS.

As was to be expected with a disease of the nature of anaplasmosis several well marked features emerge from the mass of data collected. The intensity or the degree of the changes vary from practically nil in the case of mild attacks to very marked in fatal cases, with intermediate ranges for recovery cases. The strain of *Anaplasma marginale*, which was used for all the subinoculations, was a virulent one, as is evinced by the short incubation period noted in some of the cases, the severe reactions which in three cases out of nine proved fatal and threatened with extinction several others. On the other hand, there were two very mild cases in which the only symptoms were a slight febrile reaction and the presence of a few anaplasms in the blood smears. This may have been due to the individual resistance to infection varying, or that these two animals had previously had anaplasmosis and that the reaction here merely represented a partial breakdown of immunity as a result of a heavy infection with a virulent strain. In this summary attention will be focussed on those constituents specially influenced as a sequence to this infection.

*Haemoglobin.*

This constituent of the blood shows the most noticeable alterations in amount in the direction of a decrease. In some instances the beginning of the destruction of erythrocytes would coincide with the febrile reaction, in others the decrease of the Hb. level could be noticed considerably before then. This is particularly well exemplified in Case VIII, where the Hb. had dropped from 15-11 gm. per cent. over a period of two weeks before the hyperthermia had set in.



The time interval till the minimum Hb. level is reached fluctuates considerably, e.g. in Case I, 18 days; in Case III, three weeks; in Case III, six days; in Case VI, eight days; in Case VII, ten days; in Case VIII, four weeks; and in Case IX, three weeks.

There is a distinct acceleration of destruction noted in several cases, the percentage destruction of erythrocytes per unit of time increasing towards the acme of the temperature record. Case VII shows this especially well, the Hb. content dropping from 21-12 gm. per cent. in eight days, but thereafter from 12-4 gm. per cent. within 48 hours. The mechanism of erythrolysis (or erythrorhexis) in anaplasmosis is unknown—whether it is a simple rupture of the stroma due to the penetration of the parasite into or out of the red cell, or whether an erythrolytic toxin is produced, is unknown. A large number of infected cells may be retained in the spleen and destroyed. The increased rate of destruction, however, could be explained on the normal acceleration of multiplication of the parasite. Compared with an acute redwater infection, however, erythrolysis is comparatively slow in anaplasmosis, normally no such amounts of Hb. being liberated into the plasma as to cause haemoglobinuria. The Hb. liberated is converted into bile pigments and partly excreted through the liver and urine, and partly absorbed by the tissues, giving rise to the phenomenon of icterus.

#### *Sugar.*

In cases I, II, VII, VIII, IX there exists a tendency for a slight temporary increase in the sugar concentration during the fever reaction. Case VIII shows an initial slight rise succeeded by a drop 24 hours before death.

#### *Non-Protein Nitrogen.*

The conditions found here vary according to the severity of the reaction, a marked increase was noted in all the three fatal cases (III, VI and VII), and in the severe reaction in Case II. In case III the increase towards the end was from  $\pm 20$  mgm. to 53 mgm. N per cent. and from 14-49 mgm. N per cent. in "laked" and "unlaked" respectively. In the other fatal cases VI and VII, it ranged up to 37 mgm. N per cent. and in Case II up to  $\pm 25$  mgm. N per cent. The increase only occurred during the actual febrile reaction and represents chiefly the increased U.N. Case VIII showed only a slight upward tendency, the others not accounted for remaining normal, although they were definite positive, even if mild reactors.

#### *Urea Nitrogen.*

This fraction shows the identical features as the N.P.N. and is primarily responsible for the increases mentioned above. In Case II a rise from  $\pm 5$ - $\pm 10$  mgm. per cent. is found, in Case III  $\pm 8$ - $\pm 31$  mgm. N percent., in Case VI and VII  $\pm 8$ -18 mgm. N per cent. In the others no increase or only a very slight temporary one is found. The increase is only met with during the temperature reaction, and various suggestions as to its probable origin arise, e.g. amongst others it may be mentioned that the increased metabolism associated with any rise in body temperature may be responsible; or it may be associated with protein catabolism in the circulating blood due to enzymes liberated by the protozoan; or may represent a metabolic excretion product of protozoan activity; on the other hand, the fatty degeneration of the kidneys found in severe cases of anaplasmosis, may lead to a retention as a result of renal dysfunction. Sufficient data for an explanation is as yet not available, but this fact emerges that an increase in U.N. above 15 mgm. N per cent. should be regarded as unfavourable from a prognostic point of view.

*Total Creatinine Nitrogen.*

In the severe cases a slight temporary rise can be observed: in all the other cases nothing. The range, however, does not exceed the normal limits of variations and cannot, therefore, be regarded of any special significance.

*Uric Acid Nitrogen.*

In the majority of cases a definite decrease in this fraction can be recorded, coinciding with the hyperthermia. A notable exception, however, is one fatal case in which the U.A.N. remained normal but increased 24 hours before death.

*Amino-Acid Nitrogen.*

Except in the fatal cases where a marked increase is noted before death, no definite change in either direction is observable. The increase in fatal cases is recorded only before death and figures up to 14.6 and 11.6 for "laked" and "unlaked" filtrates respectively. Although several explanations are conceivable, the assumption of hepatic dysfunction due to the severe strain thrown on this organ through the increased bile secretion, is favoured as the cause.

*Rest Nitrogen.*

A definite increase up to 14 mgm. N per cent. is only noted in the fatal cases shortly before death. The R.N. represents here probably the decomposition or metabolic products of the protozoan, and a closer investigation of this fraction should be of great interest.

*Total Nitrogen.*

Marked decreases, corresponding to the drop in haemoglobin, are encountered in all the severe or fatal cases, the degree of diminution depending primarily on the severity of the erythrolysis.

## (f) CONCLUSION.

Marked blood changes are recorded in anaplasmosis, practically every constituent being affected. The Hb. and T.N. contents drop severely, the U.A.N. also decreasing. On the other hand, striking increases are noted in the N.P.N. and the U.N., with less marked though well defined increases in the sugar level. The A.A.N. and R.N. increase shortly before death in fatal cases.

**III. PIROPLASMOSIS OF CATTLE (TEXAS FEVER, REDWATER).**

The piroplasmosis referred to here is the infection obtained with *Piroplasma bigeminum*. In connection with these researches, seven bovines were utilised, and after several analyses of the normal blood had been performed in each case, they were injected with infective blood. In only three cases was a reaction obtained, but with the exception of the one case given here, they were so mild, that they could be regarded as virtually non-reactors. An occasional rare parasite was found in the blood smears, and a slight undulation in the temperature record. Analyses during this period showed slight blood changes which were in the same general direction as those recorded here in Table I. It was then decided to repeat this experiment in the near future with a larger number of animals, and only to submit in the meantime the data in respect of the one typical severe case recorded here, primarily for comparison and contrast with the figures obtained in connection with anaplasmosis.



CHEMICAL BLOOD STUDIES V.

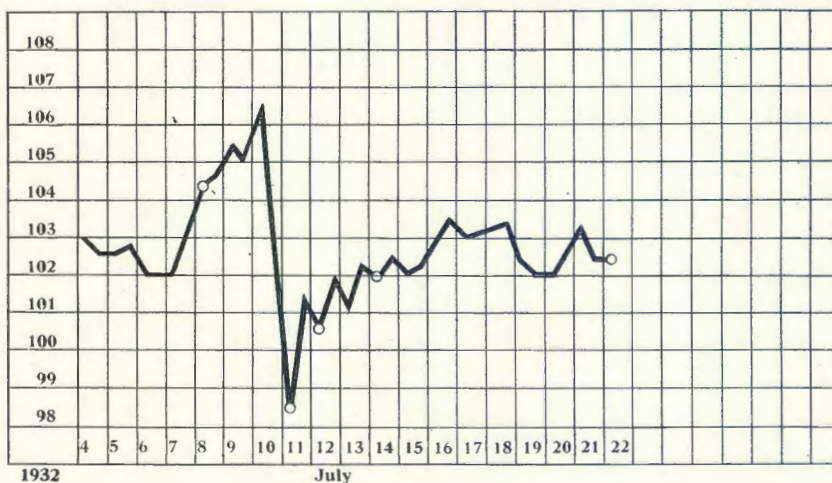
In a severe case of piroplasmosis the destruction of erythrocytes is so rapid that the liberated haemoglobin is partly excreted as such through the kidneys, resulting in haemoglobinuria, partly converted into bile pigments, as is evinced by the icterus generally associated with this infection. In the present case there are, as would be expected, several blood changes from the chemical composition point of view, e.g. such as the *rapid* drop from the normal Hb. level even if compared with anaplasmosis in which erythrorhexis is also marked; the striking increase in the sugar content and N.P.N. It is felt, however, that a detailed comparison with anaplasmosis would be more justified when a larger number of cases are available and this aspect is therefore not dealt with at present. The normal data have, however, been utilised in drawing up the "normal ranges" as given under Anaplasmosis.

(a) EXPERIMENTAL DATA.

CASE I.

B. 3198. *Redwater. Treated and Recovered.*

*Temperature Chart I.*



*History:* B. 3198. A two year old heifer in good condition. Inoculated against anthrax and blackquarter in November, 1930, the anthrax immunity being tested 12 months later. During May-June, 1932, it passed through a severe *Anaplasmosis* reaction (*vide*). On 28/6/32 it received subcutaneously 75 c.c. blood from B. 3735. The parasites (*P. bigeminum*) were first noticed 8/7/32, coinciding with the onset of the temperature reaction. Occasional *A. marginale* reappeared, but the *P. bigeminum* were very numerous and the symptoms, including severe haemoglobinuria, so pronounced that from a practical point of view the presence of the *A. marginale* can be overlooked. This patient received chemotherapeutic treatment (10/7/32), the temperature dropping as a result from 105.6-97.8 per cent. within 24 hours. Prior to the injection the animal was down, showed salivation, icterus, anaemia and dyspnoea, and from a prognostic point of view unlikely to recover.

TABLE I.  
B. 3198. *Redwater* (*P. bigeminum*). *Treated (Recovered)*.

| Date.....              | 1/7/32.            | 8/7/32.        | 11/7/32.       | 12/7/32.         | 14/7/32.            | 22/7/32.       |
|------------------------|--------------------|----------------|----------------|------------------|---------------------|----------------|
| Time.....              | —                  | —              | —              | —                | —                   | —              |
| Temp. Reaction.....    | P.I.N.             | R.             | R.             | R.               | N.                  | N.             |
| Haemoglobin gm. %..... | 10·35              | 8·88           | 3·58           | 3·42             | 5·18                | 9·60           |
| Sugar mgm. %.....      | L 60·24<br>U 53·76 | 66·67<br>59·52 | 83·33<br>77·52 | 158·72<br>147·12 | 76·34<br>68·03      | 60·61<br>52·63 |
| Total N. gm. %.....    | 2·500              | 2·220          | 1·548          | 1·660            | 1·562               | 2·444          |
| N.P.N. mgm. %.....     | L 13·26<br>U 10·38 | 12·50<br>9·37  | 29·42<br>27·21 | 41·84<br>38·48   | 19·86<br>16·58      | 16·72<br>11·00 |
| Coag. N. gm. N %..     | L 2·487<br>U 2·490 | 2·207<br>2·211 | 1·519<br>1·521 | 1·618<br>1·622   | 1·542<br>1·545      | 2·426<br>2·433 |
| Urea mgm. N %... L     | 3·35               | 4·00           | 12·42          | 26·60            | 9·75                | 3·84           |
| "    U %... L          | 7·14               | 8·40           | 26·04          | 55·86            | 20·58               | 8·00           |
| mgm. N %... U          | 3·00               | 3·65           | 12·07          | 26·58            | 9·31                | 4·00           |
| "    U %... U          | 6·30               | 7·77           | 25·41          | 55·86            | 19·53               | 8·40           |
| Total Creatinine       |                    |                |                |                  |                     |                |
| mgm. N %... L          | 2·04               | 2·01           | 3·48           | 2·86             | 2·35                | 2·27           |
| "    TC %... L         | 5·50               | 5·40           | 9·40           | 7·72             | 6·36                | 6·16           |
| mgm. N %... U          | 1·44               | 1·75           | 3·48           | 2·50             | 2·01                | 1·86           |
| "    TC %... U         | 3·86               | 4·70           | 9·10           | 6·74             | 5·40                | 5·02           |
| Uric acid              |                    |                |                |                  |                     |                |
| mgm. N %... L          | 0·33               | 0·33           | 0·24           | 0·37             | 0·33                | 0·35           |
| "    UA %... L         | 0·99               | 1·00           | 0·73           | 1·12             | 1·00                | 1·04           |
| mgm. N %... U          | 0·20               | 0·21           | 0·22           | 0·29             | 0·24                | 0·24           |
| "    UA %... U         | 0·59               | 0·64           | 0·67           | 0·87             | 0·73                | 0·73           |
| Amino acid             |                    |                |                |                  |                     |                |
| mgm. N %... L          | 5·00               | 4·40           | 8·75           | 8·33             | 4·30                | 4·70           |
| "    U %... U          | 3·68               | 2·59           | 9·09           | 6·73             | 3·04                | 2·54           |
| Rest Nitrogen          |                    |                |                |                  |                     |                |
| mgm. N %... L          | 1·54               | 0·76           | 3·73           | 2·68             | 2·05                | 4·56           |
| "    U %... U          | 1·06               | 0·17           | 1·35           | 1·38             | 1·98                | 1·36           |
| Plasma.....            | n.u.               | icteric        | icteric        | icteric          | slightly<br>icteric | n.u.           |

*Main Features of Analytical Data.*

*Hb.*—This is initially on the low side, the animal not having recovered completely from its previous anaplasmosis infection. With the appearance of the redwater parasites there is a very rapid drop in Hb. level from 8·8–3·6 gm. per cent. within 72 hours. This rapid destruction was reflected in the severe haemoglobinuria which occurred. Regeneration was remarkably rapid, the original level being nearly reached in about ten days' time.

*Sugar.*—Shows a marked rise during the reaction from 66–158 mgm. per cent, and from 60–147 mgm. per cent. in "laked" and "unlaked" filtrates respectively.

*N.P.N.*—An increase from  $\pm$  12–42 mgm. N per cent. and from  $\pm$  10–38 mgm. N per cent. in "laked" and "unlaked" filtrates respectively.

*U.N.*—Shows similar features—an increase from 4–27 mgm. N per cent. taking place.

*T.C.N.*—Is highest during the reaction for both filtrates.

*A.A.N.*—This increases from  $\pm$  4·5–8·5 mgm. and from  $\pm$  3–9 mgm. for "laked" and "unlaked" filtrates respectively, the acme being reached during the febrile reaction.

*T.N.*—A drop from 2·5–1·5 gm. per cent. is recorded, coinciding with the decrease in the Hb. level.

*R.N.* and *U.A.N.*—Nothing unusual.



## (b) SUMMARY.

Only one case being available no general deductions as to the changes in the blood resulting from a *P. bigeminum* infection are permissible. Summarising the above case one notices marked increases in nearly all the nitrogenous fractions and in sugar, associated with the period of maximum erythrocyte destruction. The efficacy of the kidneys renders a rapid excretion of all "free" haemoglobin possible, but apparently the excretion of urea is retarded temporarily, leading to an increase in the blood for 2-3 days. The rise in A.A.N. is peculiar, particularly if it be remembered that the animal ceased feeding just during the period in which the A.A.N. was highest. Conceivably the increase is due to the protein decomposition rather than an interference with the absorption from the intestines and deamination of amino-acids in the liver. On the other hand the liver has a severe strain placed on it by the secretion of abnormally large amounts of bile pigments as a result of the excessive erythrocyte destruction and deamination may thereby be temporarily interfered with. If this is the correct interpretation the increase in urea would find its explanation not in increased formation, but in retention or retarded excretion through the kidneys, possibly due to degenerative changes. More research is necessary before any definite *modus vivendi* for the various observations can be formulated and substantiated. In the meantime the case is of interest in so far as it indicates some of the changes in composition resulting during a fulminant severe erythrolysis.

## IV. ACKNOWLEDGMENTS.

In conclusion I wish to record my appreciation of and indebtedness to my colleagues Messrs. W. O. Neitz and B. S. Parkin for their co-operation in permitting me to bleed their experimental animals, whenever required and for access to their records; also to Mr. W. F. Averre and assistants for performing the bleeding.

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